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厦门大学

博 士 学 位 论 文

多氯联苯对小鼠附睾的毒性效应及机制的研究

Effects and mechanisms of polychlorinated biphenyls on mouse epididymis

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摘　要

多氯联苯（Polychlorinated biphenyls，PCBs）是最难以降解的持久性有机污染物之一。有关 PCB 的生殖毒性效应及机制已开展了较多的研究，但 PCB 对附睾的毒性效应及机制还不清楚。本研究以 C57BL/6 品系青春期雄性小鼠为研究对象，以商品化的 Aroclor 1254 为 PCB 代表，研究环境水平的 PCB 暴露（0.5、5、50 和 500 μg/kg）对附睾的毒性效应及机制。

青春期雄性小鼠在 Aroclor 1254 暴露 50 天后，其体重、睾丸和附睾重量没有显著性变化，精子密度、精子活力以及顶体酶活性呈现剂量依赖性下降，精子畸形率呈现剂量依赖性上升。血清中的雌激素水平表现出剂量依赖性的下降，雄激素的水平没有表现出显著性的变化。Western blot 检测的结果显示：附睾中的雌激素受体 α（Estrogen receptor α，ERα）和雌激素受体 β（Estrogen receptor β，ERα）并无显著性变化，而雄激素受体在 500 μg/kg 组表现出显著性下降，提示 Aroclor 1254 对附睾表现出抗雄激素效应。

利用 cDNA 微阵列（Microarray）技术研究附睾头部在低浓度（5 μg/kg）和高浓度（500 μg/kg）Aroclor 1254 暴露下基因的差异表达，结果显示：共筛选出 2350 个差异表达基因，其中低浓度组引起附睾头部 572 个基因的差异表达，包括上调基因 212 个，下调基因 360 个；高浓度组引起附睾头部 1769 个基因的差异表达，其中上调基因 433 个，下调基因 1336 个。应用实时荧光定量 PCR（Real-time fluorescent quantitative PCR，Real-time PCR）对部分差异表达基因进行了 mRNA 水平的验证，结果证明了芯片检测的可靠性。进一步应用分子注释系统（Molecule Annotation System，MAS），结合功能注释聚类（Functional Annotation Clustering）以及聚类分析（Clustering）等生物信息技术，对差异表达基因信息和功能以及所参与的信号通路进行挖掘和注释，得到差异基因中最为显著的功能类别和信号通路，其中涵盖了 GTP 结合、核小体组装、能量代谢、RNA 加工等方面。为进一步探索 PCB 对附睾的毒性效应机制提供了依据。

根据功能注释聚类的结果，发现与 GTP 结合相关的基因在高浓度 Aroclor 1254 暴露后表现出最高的富集度（Enrichment score），表明相关基因的表达变化

是 Aroclor 1254 暴露作用于附睾表达谱的最显著的效应之一。MAS 分析表明相关基因的功能包括了紧密连接和肌动蛋白细胞骨架的调控。Western blot 检测表明相关的小 GTP 结合蛋白，如 K-ras、Cdc42 以及 RhoA 等的表达水平在 PCB 暴露后表现出显著性的下调。小 GTP 结合蛋白通过调节肌球蛋白调节轻链（Myosin regulatory light chain，MRLC）和丝切蛋白（Cofilin）的磷酸化水平来调节紧张纤维的收缩和肌动蛋白聚合的稳定性。Western blot 结果表明 MRLC 磷酸化水平上升而 Cofilin 磷酸化水平下降，提示紧张纤维的收缩加强、肌动蛋白聚合的稳定性增加。

小 GTP 结合蛋白表达的变化和纤维的收缩加强提示紧密连接功能的改变。Western blot 结果表明紧密连接的组成蛋白（胞质紧密粘连蛋白 1）ZO-1 在 500 μg/kg PCB 暴露下表现出显著性的下调。免疫组化结果表明定位与细胞顶端连接的 ZO-1 染色并没有明显的变化。免疫共沉淀的结果表明，Occludin 的酪氨酸磷酸化的水平上升。电镜下的硝酸镧示踪提示紧密连接的透过性增加。紧密连接的透过性关系到精子成熟环境的维持，PCB 暴露对紧密连接的影响提示了 PCB 对生殖毒性的新机制。

对低浓度和高浓度都能够显著性诱导的差异基因进行基因分类学注释（Gene Ontology，GO），选取 GO 注释包括细胞外空间（Extracelluar space 或 Extracelluar region）为候选标志物。从中选取鞘脂激活蛋白原（Prosaposin，Psap）进行蛋白表达水平的验证。将 Psap 表达水平与精子指标和肝脏中的 PCB 浓度建立相关性，结果表明 Psap 表达与 PCB 浓度以及精子畸形率负相关而与精子活力正相关。提示 Psap 可能作为 PCB 暴露和雄性生殖毒性的候选标志物。

关键词：多氯联苯，附睾，生殖毒性

ABSTRACT

Polychlorinated biphenyls (PCBs) are among the most persistent environmental pollutants and the reproductive toxicity of PCBs has been extensively studied. However, the toxicological effects and mechanisms of PCBs on epididymis remain unclear. In the present study, toxicological effects and mechanisms of PCB exposures of environmental levels on epididymis of purbertal C57BL/6 mice were investigated.

Male purbertal C57BL/6 mice were exposed to Aroclor 1254, a commercial PCB mixture once widely used by gavage at low dose (0.5, 5, 50 and 500 μg/kg/3days. respectly). After exposure for 50 days, the epididymal sperm count, sperm motility and acrosin activity showed a dose-dependent decrease, and the sperm abnormality showed a dose-dependent increase, while no changes in body weight, testis weight or epididymis weight were observed. Serum estrogen level decreased in a dose-dependent manner and the alteration of serum androgen level remained insignificant. The results of Western blot analysis demonstrated that the expression of androgen receptor (AR) in caput epididymis were significantly decreased in 500 μg/kg group while the expression of estrogen receptor α (ERα) and estrogen receptor β (ERβ) remained unchanged. It suggested an antiandrogen effect of Aroclor 1254 on epdidymis in low-dose PCB exposure.

cDNA microarray technique was used to investigate the gene expression profiles of caput epididymis in response to low dose (5 μg/kg) and high dose (500 μg/kg) Aroclor 1254 exposure and 2350 differential expressed genes identified. After exposed to low dose Aroclor 1254, 572 genes were differentially regulated, which included 212 up-regulated genes and 360 down-regulated genes. After exposed to high dose Aroclor 1254, 1769 genes were differentially regulated, which included 433 up-regulated genes and 1336 down-regulated genes. Real-time fluorescent quantitative PCR (qPCR) were performed on selected genes for the confirmation of microarray data. The results were in agreement with the microarray data.

Bioinformatics tools including functional annotation clustering and molecule annotation system (MAS) were applied to reveal the biological significance of the gene expression data. The differential expressed genes were involved in a variety of function categories and biological pathways, such as GTP binding, nucleosome assembly, energy metabolism, RNA processing etc.

The results of functional annotation clustering indicated that the functional category related to GTP binding showed the highest enrichment score among the functional categories affected by 500 μg/kg Aroclor 1254 exposure. It suggested that changes in the expression of genes related to GTP binding are among the most significant effects of Aroclor 1254 on epididymis. MAS analysis indicated that GTP binding proteins may involved in many important biological processes, such as regulation of actin cytoskeleton and assembly of tight junction. Western blot analysis revealed significant decreases in protein abundance of small GTP binding proteins: Cdc42, Ras and RhoA after PCB exposure. Alteration in these small GTP binding proteins may affect the contraction of stress fiber and stability of actin filament by affecting the phosphorylation status of their down stream effectors: myosin regulatory light chain (MLC) and cofilin. The increase of phosphorylated MLC and decrease of phosphorylated cofilin were detected by western blot. These results suggested an increase in stress fiber contraction and a decrease in actin filament stability.

The GTP binding protein related actin cytoskeleton alteration may suggest a disruption on tight junction function. Western blot indicated a signifiant decrease in ZO-1 expression and a trend of decrease in occludin expression. Immunohistochemistry showed a disruption of apical occludin expression and no observable changes in apical ZO-1 expression. Immunoprecipitation assay showed an increase in tyrosine phosphorylated occludin. Lanthanum nitrate tracing under the electron microscope revealed an increased permeability of apical tight junction after 500 μg/kg Aroclor 1254 treatment. The apical tight junction permeability in epididymal principal cells is important to the maintenance of microenvironment which supports the sperm maturation. The disruption on epididymal tight junction may be involved in a novel mechanism by which PCBs affect the post-testicular

sperm maturation.

Gene ontology (GO) annotations were performed on differential expressed genes which were both regulated by 5 μg/kg exposure and 500 μg/kg exposure. The genes related to the GO term: extracelluar space or extracelluar region were selected as biomarker candidates. Among these candidates, the protein abundance of prosaposin (psap) was examined in epididymis and sperm by western blot and showed a dose-dependent decrease. The expression level of psap in sperm was negatively correlated to hepatic PCB accumulation and sperm abnormality and was positively correlated to sperm motility. The results suggested that psap is potential biomarker of PCB male reproductive toxicity.

Key words: Polychlorinated biphenyls; Epididymis; Reproductive toxicity